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Specification and Drawing, as originally filed, with Application for Patent Serial No: **2,413,240**, on November 29, 2002, by **MCN BIOPRODUCTS INC.**, assignee of Rex W. Newkirk, David D. Maenz and Henry L. Classen, for "Purification of Inositol from Plant Materials".

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ABSTRACT

Discloses a process for production of inositol, which is a highly valued B-vitamin, from plant material. Inositol is present in the plant start material as a phytate, which is the storage form for phosphorus in the plants. A phytase enzyme is introduced to an aqueous slurry of plant material to partially hydrolyse myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) to generate inositol phosphates (myoinositol penta phosphate (IP5) myoinositol tetra phosphate (IP4), myoinositol triphosphate (IP3), myoinositol diphosphate (IP2) and myoinositol monophosphate (IP1) but without substantial hydrolysis of myoinositol 1-phosphate IP1). The insoluble materials are filtered from the slurry output and the charged ionic species of the solution are recovered into an ionic fraction. The inositol phosphates in the ionic fraction are fully hydrolized, by process parameters of pressure temperature and pH or an enzyme such as acid phosphatase. The charged ionic species of the solution are removed leaving a neutral inositol enriched fraction. The inositol in the inositol enriched fraction solution can be concentrated, crystallized and dried to form a final dry inositol product.

Purification of Inositol from Plant Materials

Field of the Invention

This invention relates to production of inositol from plant materials.

Background to the Invention

Inositol is a highly valued B-vitamin. Plants contain inositol within the structure of phytate (myoinositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate), which is the storage form for phosphorus in the plants.

Purification of inositol from a plant material requires conversion of the phytate to inositol and purification from other components in the plant starting material.

Purification of inositol from an aqueous slurry of plant material is difficult. One approach is to hydrolyze the phytate to inositol in the slurry. However, inositol is a neutral soluble sugar that is very similar in molecular size and charge characteristics to other sugars such as glucose that are often present in high levels in plant materials. As such it is problematic to separate the inositol from similar carbohydrates in the slurry.

Another approach to production of inositol from plant materials is to purify the phytate from the starting slurry and hydrolyze the purified phytate to inositol in a latter step in the overall process. However, phytate in plants exists as phytin which is an insoluble complex of phytate with minerals such as Ca, Mg and K. Direct phytate purification from an aqueous slurry of plant materials will require solubilization of phytin and then separation of the phytate from the remainder of the components of the slurry. Efficient extraction, solubilization of phytin and separation from the remaining components of the slurry is difficult.

Description of the Invention

This invention describes a useful and novel process for overcoming the inherent difficulties in inositol purification from plant materials. In accordance with the inventive process, phytate in an aqueous slurry of vegetable material, is partially hydrolyzed by incubating the slurry with an enzyme product enriched in phytase.

The phytase enzyme can hydrolyze phytate, inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3) and inositol diphosphate (IP2); however, the phytase has little activity for hydrolysis of inositol monophosphate (IP1). Acid phosphatase can readily hydrolyze IP1 to free inositol. As such a source of phytase containing little or no acid phosphatase can be used to partially hydrolyze the phytate to IP5, IP4, IP3, IP2 and IP1. A source of phytase containing acid phosphatase activity can also be used, however, it is essential that conditions of the reaction greatly favour phytase activity to avoid substantial hydrolysis of IP1 by acid phosphatase. In using a source of phytase containing acid phosphatase the preferred pH of the reaction is greater than 3.0 and less than 7 for optimum phytase activity without substantial hydrolysis of IP1 to inositol.

IP5, IP4, IP3, IP4, IP2 and IP1 as the major products of the reaction, are highly soluble negatively charged compounds that will exist in solution in the partially hydrolyzed slurry. The partially hydrolyzed slurry is separated by physical separation means, such as filtration or centrifugation, to generate an inositol phosphate containing soluble fraction (C - total soluble fraction) and an insoluble fraction (D - insoluble fraction). Unlike inositol, inositol phosphates retain a negative charge. The total soluble fraction is then separated into a subfraction enriched in ionic constituents (including inositol phosphates) of the total soluble fraction (E - ionic fraction 1) and a second subfraction enriched in neutral soluble constituents of the total soluble fraction (F - neutral fraction). These fractionation steps are completed using known techniques for the separation of charged ionic species from soluble neutral compounds in the solution. The next step in the process is to complete the hydrolysis of inositol phosphates in the ionic fraction. This process can be done with enzymes or without enzyme-based catalysis

under controlled conditions of temperature, pressure and pH. In using enzymes the preferred approach is to use an enzyme source containing acid phosphatase at a pH of less than 4 for optimum activity. Complete hydrolysis of inositol phosphates will generate neutral inositol in the ionic fraction (G). Inositol can be readily separated from the remainder of the soluble compounds in the ionic fraction using known techniques for separating charged from neutral compounds in solution. This process generates a second ionic fraction (H - anionic fraction 2) enriched in ionic constituents in the total soluble fraction and a neutral inositol fraction (I - inositol fraction). Inositol in the inositol fraction can be concentrated, crystallized and dried to form a final dry purified inositol product.

Brief Description of the Drawings

Figure 1 is a process flow chart depicting processing stages in accordance with the invention.

Detailed Description of the Preferred Embodiments

Figure 1 shows a process flow chart of the various steps in the process of the invention. In the process, a slurry of plant start material is processed to produce a neutral inositol product. At the outset of the process, an aqueous slurry of plant vegetable material (A) is incubated with an enzyme product enriched in phytase to hydrolyze the phytate component of the slurry.

The preferred phytase enriched enzyme can hydrolyze phytate, inositol pentaphosphate (IP5), inositol tetraphosphate (IP4), inositol triphosphate (IP3) and inositol diphosphate (IP2), but has little activity for hydrolysis of inositol monophosphate (IP1). Acid phosphatase can readily hydrolyze IP1 to free inositol, consequently, a source of phytase containing little or no acid phosphatase can be used to partially hydrolyze the phytate to IP5, IP4, IP3, IP2 and IP1. A source of phytase containing acid phosphatase activity can also be used, however, if such a source of phytase is used, it is essential that conditions of the reaction greatly favour phytase activity that avoids substantial hydrolysis of IP1 by the acid phosphatase. When using a source of phytase containing acid

phosphatase, the preferred pH of the reaction is greater than 3.0 and less than 7 for optimum phytase activity without substantial hydrolysis of IP1 to inositol.

IP5, IP4, IP3, IP2 and IP1 as the major products of the reaction, are highly soluble negatively charged compound that will exist in solution in slurry B.

Slurry B can then be separated, by physical separation means, such as filtration or centrifugation, to generate an inositol phosphate containing soluble fraction (C - total soluble fraction) and an insoluble fraction (D - insoluble fraction). Unlike inositol, inositol phosphates retain a negative charge. The total soluble fraction C is then separated into a subfraction enriched in ionic constituents (including inositol phosphates) of the total soluble fraction (E - ionic fraction 1) and a second subfraction enriched in neutral soluble constituents of the total soluble fraction (F - neutral fraction). These fractionation steps are completed using techniques for the separation of charged ionic species from soluble neutral compounds in the solution.

The next step in the process is to complete the hydrolysis of inositol phosphates in the ionic fraction. This process can be done with enzymes or without enzyme-based catalysis under controlled conditions of temperature, pressure and pH. In using enzymes the preferred approach is to use an enzyme source containing acid phosphatase at a pH of less than 4 for optimum activity. Complete hydrolysis of inositol phosphates will generate neutral inositol in the ionic fraction (G). The neutral inositol can be readily separated from the remainder of the soluble compounds in the ionic fraction using known techniques for separating charged from neutral compounds in solution. This separation process generates a second ionic fraction (H - anionic fraction 2) enriched in ionic constituents in the total soluble fraction and a neutral inositol fraction (I - inositol fraction). Inositol in the inositol fraction can be concentrated, crystallized and dried to form a final dry purified inositol product.

The embodiments of invention in which an exclusive property or privilege is claimed are defined as follows:

1. A process for producing inositol from vegetable materials comprising the steps of:

(a) slurrying a vegetable material in water in the presence of a phytase enzyme to partially hydrolyze phytic acid but to avoid substantial hydrolysis of inositol monophosphate;

(b) separating the insoluble fraction from said slurry to produce a soluble fraction;

(c) fractionating said soluble fraction to recover an ionic fraction;

(d) hydrolysing the inositol phosphates in said ionic fraction; and

(e) fractionating the hydrolyzed ionic fraction to recover a neutral fraction enriched in inositol.

2. The process of claim 1 wherein said phytase enzyme does not include acid phosphatase.

3. The process of claim 1 wherein said step of slurrying the vegetable material is carried out at a pH between about 3.0 and about 7.0.

4. The process of claim 3 wherein said phytase enzyme includes acid phosphatase.

5. The process of claim 1 wherein said step of separating the insoluble fraction is carried out by centrifugation.

6. The process of claim 1 wherein said step of separating the insoluble fraction is carried out by filtration.

7. The process of claim 1 wherein said step hydrolysing the inositol monophosphate in the ionic fraction is performed by hydrolysing the ionic fraction in the presence of a second phytase enzyme.

8. The process of claim 7 wherein said second phytase enzyme includes acid phosphatase.

9. The process of claim 1 wherein said step of slurrying the ionic fraction to substantially hydrolyse the inositol monophosphate contained therein is performed without enzyme under controlled conditions of temperature, pressure and pH.

Fig1. Purification of inositol from plant materials